Towards a greater understanding of the presence, fate and ecological effects of microplastics in the freshwater environment

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CHAPTER 5

Acute toxicity of organic pesticides to *Daphnia magna* is unchanged by co-exposure to polystyrene microplastics

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Abstract

Daphnia magna were exposed to two pesticides in the presence or absence of microplastics (300 000 particles ml^{-1} 1 μm polystyrene spheres) and to microplastics alone. The pesticides were dimethoate, an organophosphate insecticide with a low log Kow, and deltamethrin, a pyrethroid insecticide with a high log Kow. *Daphnia* were exposed to a nominal concentration range of 0.15, 0.31, 0.63, 1.25, 2.5, 5 mg l^{-1} dimethoate and 0.016, 0.08, 0.4, 2, 5 and 10 μg l^{-1} deltamethrin. Exposure to polystyrene microplastics alone showed no effects on *Daphnia magna* survival and mobility over a 72 hour exposure. In the dimethoate exposures, mobility and survival were both affected from a concentration of 1.25 mg l^{-1}, with effects were seen on mobility from 28 hours and survival from 48 hours, with greater effects seen with increasing concentration and exposure time. In deltamethrin exposures, survival was affected from a concentration of 0.4 μg l^{-1} and mobility from a concentration of 0.08 μg l^{-1}. Effects of deltamethrin on mobility were seen from 5 hours and on survival from 28 hours, with greater effects on survival and mobility seen with increasing concentration and exposure time. Contrary to expectations, pesticide toxicity to *Daphnia magna* was not affected by the presence of microplastics, regardless of chemical binding affinity (log Kow). This therefore suggests that polystyrene microplastics are unlikely to act as a significant sink, nor as a vector for increased uptake of pesticides by aquatic organisms.

1. Introduction

Microplastics are a pollutant of increasing environmental concern based on their ubiquitous and persistent nature. It is widely recognised that microplastics will form biological and chemical associations within the environment. For example microplastics may become associated with algae or bacteria (biofilms) (Hoellein et al., 2016; McCormick et al., 2014) or may sorb organic chemicals due to their hydrophobic nature (Bakir et al., 2012; Koelmans et al., 2016; Mato et al., 2001). The potential for association of hydrophobic organic chemicals (HOCs) with microplastics has been recognised and has prompted studies on whether this association will affect the bioavailability of HOCs, and thus their toxicity to organisms. Studies have shown that microplastics can make HOCs either more bioavailable, by acting as a vector for uptake following ingestion (Avio et al., 2015a; Chen et al., 2017; Rochman et al., 2013c), or less bioavailable due to strong irreversible binding of HOCs to microplastics, removing HOCs from solution and remaining bound even if ingested (Beckingham and Ghosh, 2016). It has even been suggested that microplastics may lead to the removal of HOCs from body tissues following the ingestion of clean plastics by a previously contaminated organism (Koelmans et al., 2013). The majority of studies on microplastics and chemical associations to date have focussed on the marine environment. However, concentrations of HOCs and microplastics in continental terrestrial and freshwater environments are expected to be higher than marine environments due to their proximity to the sources combined with limited dispersal and dilution, thus highlighting the importance of studying terrestrial and freshwater systems (Dris et al., 2015b; Horton et al., 2017b).

The capacity for a chemical to bind to microplastics is, among other factors, determined by its hydrophobicity, usually expressed as the log Kow value. Kow represents the partition coefficient between octanol and water (Brooke, 2014). A chemical with a high log Kow will have a lower water solubility than less hydrophobic substances (with a lower log Kow), meaning that it will preferentially bind to organic particulate matter within the system rather than remaining within solution (Lee et al., 2014; Mackay et al., 1980). It is therefore expected that a chemical with a high log Kow (high hydrophobicity) will also have a higher affinity for binding to microplastics in an aqueous system than a chemical with a lower log Kow (higher hydrophilicity) (Wang et al., 2018b). Such interactions can potentially remove the chemical from solution and concentrate it on the surface of the plastic, thereby changing bioavailability (Gouin et al., 2011; Lee et al., 2014; Velzeboer et al., 2014). The aim of this study was therefore to investigate how the presence of microplastics would affect the toxicity of high and low log

Kow organic pesticides to a relevant freshwater organism, the cladoceran *Daphnia magna*. Pesticides were chosen as their toxicity is well-documented. The starting hypothesis was that the presence of microplastics within an aquatic solution would reduce the toxicity of a pesticide with a high log Kow, due to its high binding capacity to the microplastics making it less bioavailable (Beckingham and Ghosh, 2016; Koelmans et al., 2013), whereas the toxicity of a low log Kow pesticide would be less affected by the presence of microplastics.

2. Materials and methods

2.1. The test chemicals

We chose two pesticides to represent chemicals with high and low log Kow, both with known toxicity to *Daphnia magna*. Dimethoate and deltamethrin were chosen both for their differing chemical properties (specifically log Kow) and because they are environmentally relevant, being representative of two widely used classes of insecticides. Both pesticides target receptors associated with nervous system function to cause neurotoxicity. Dimethoate is an organophosphate insecticide with a low log Kow (0.704) (Pesticide Properties Database, 2017b). It is relatively soluble in water (between 23.5-39.8 g l^{-1} at 25°C) (Pesticide Properties Database, 2017b; Sigma-Aldrich, 2017). It was first registered for use in 1962 and is still widely applied to agricultural land worldwide (Van Scoy et al., 2016). Deltamethrin is a pyrethroid insecticide also widely used in agriculture (Ren et al. 2009) and aquaculture (Ernst et al. 2014). Deltamethrin is very poorly soluble in water, with a solubility between 0.2-2 µg l^{-1} at 25°C (Mestres and Mestres, 1992; Pesticide Properties Database, 2017a). Due to this hydrophobic nature, with a log Kow reported between 4.6 (Kaneko, 2010) and 6.2 (PubChem Compound Database, 2017), deltamethrin entering a water body would be expected to adsorb readily to particulate matter such as microplastics, in addition to sediment and organic matter (Lee et al., 2014; Lee et al., 2002).

2.2. The test organism

Daphnia magna is commonly used for ecotoxicological testing and as such, toxicity data are readily available for *D. magna* for both deltamethrin and dimethoate toxicity (Andersen et al., 2006; Toumi et al., 2013), as well as information on microplastic uptake and toxicity (Besseling et al., 2014; Jemec et al., 2016; Rehse et al., 2016). This makes them an ideal species for

investigating how toxicity may be influenced by the interaction of these pesticides with microplastics.

D. magna were taken from the Leiden University culture which has been continuously maintained for over six years in the laboratory. According to the OECD guideline 202, *D. magna* were cultured in glass containers with Artificial ElendtM4 medium at a density of 1 individual/10 ml of ElendtM4 medium (OECD, 2004). The culture medium was refreshed twice a week. The test organisms were fed *ad libitum* with *Raphidocelis subcapitata* algae and maintained inside a temperature-controlled chamber (20 ± 1 °C) under a 16:8 light-dark cycle. Throughout the duration of culturing, sensitivity of the test species was checked every six months using the standardized toxicity test conducted with $K_2Cr_2O_7$ as a reference compound (OECD, 2004).

2.3. Preparation of the microplastic beads

Microplastics as fluorescent polystyrene beads were purchased from Phosphorex (USA) with a nominal size of 1 µm, as a solution containing DI water, an anti-microbial agent (sodium azide) and a surfactant (Tween 20). The size of particles was confirmed by TEM as being 1.2 ± 0.2 µm (mean ± SD) (Fig S1). Previous experimental studies have shown that microplastics within the size range 20 nm – 5 µm are commonly ingested by *D. magna*, as they represent a similar size range as their common algal food sources (Besseling et al., 2014; Ogonowski et al., 2016; Rehse et al., 2016; Rist et al., 2017; Rosenkranz et al., 2009). Both sodium azide and Tween 20 may act as toxicants and so the beads were washed in order to remove these from the solution used for microplastic spiking. For washing, the supplied stock of beads (1 ml) was diluted to approximately 12 ml with Milli-Q water, vortexed to mix and then centrifuged at 5180 g (5000 rpm) (Beckman Coulter Avanti J-E centrifuge, USA) for 5 minutes. The supernatant was then carefully pipetted leaving approximately 1 ml of solution containing the particles at the bottom. These cleaning steps of dilution and centrifuging were then repeated twice more to ensure maximum removal of the sodium azide and Tween20. Following the final cleaning step the solution was diluted with Milli-Q water to give a total stock solution volume of 10 ml. The number of beads per ml of this new bead stock was measured using a flow cytometer (BD Accuri C6, BD Biosciences, USA). This bead stock was used for spiking the test medium to a nominal concentration of 300 000 particles ml^{-1}. This concentration is roughly equivalent to the number of algal cells that daphnids would be exposed to in an excess food

situation (*i.e.* under culture conditions) and equates to approximately 0.29 µg ml^{-1} (287.7 µg l^{-1}, calculations in SI).

2.4. Preparation of the test solutions

A dimethoate (PESTANAL®, analytical standard, Sigma Aldrich Ltd, UK) stock solution of 1 g l^{-1} was prepared directly in Elendt artificial freshwater. In order to produce the required concentrations, the appropriate amount of stock solution was made up to 250 ml with Elendt artificial freshwater. Based on toxicity values of dimethoate to *D. magna*, with 48 h LC_{50} ranging from 0.86-2 mg l^{-1} (Beusen and Neven, 1989; Syberg et al., 2008), exposure concentrations were made in the range 0.156, 0.313, 0.625, 1.25, 2.5, 5 mg l^{-1} (0.68, 1.36, 2.73, 5.45, 10.9, 21.8 µM) .

To spike the test medium with deltamethrin it was necessary to dissolve it in a solvent carrier due to its low solubility in water. Deltamethrin (certified reference material, Sigma-Aldrich Ltd, UK) was dissolved in acetone to prepare a stock solution of 10 000 µg l^{-1}. A serial dilution of this stock, was made by further dilution in acetone to create a deltamethrin concentration series for spiking into artificial freshwater. A volume of 375 µl of the relevant stock was added to 250 ml Elendt artificial freshwater (giving an acetone concentration of 0.15 % within the exposure solution) in order to give the required exposure concentration range: 0.016, 0.08, 0.4, 2, 5 and 10 µg l^{-1} (0.03, 0.16, 0.79, 3.96, 9.9, 19.79 nM). These exposure concentrations were based on literature toxicity data for *D. magna* with 48 h LC_{50}s ranging from 0.038-0.45 µg l^{-1} (Ren et al., 2009; Xiu et al., 1989) and 24 h LC_{50}s ranging from 0.113-9.4 µg l^{-1} (Toumi et al., 2013; Xiu et al., 1989).

For both pesticides, treatments were prepared with and without microplastics. For the microplastic treatments, the polystyrene bead stock solution was added to the exposure solutions after the artificial freshwater had been spiked with the chemicals. The appropriate volume of stock solution (as determined using the flow cytometer) was added to a volume of 250 ml of spiked solution to give a nominal concentration of 300 000 particles ml^{-1}. Four replicates of 40 ml exposure solution held in 50 ml glass jars were prepared for each treatment. With an average particle size of 1.2 µm ± 0.2 µm, the average surface area of the microplastics within 40 ml was calculated as approx. 38-74 cm^2 dependent on variation in particle size (surface area calculations are in SI). This concentration of particles provides a comparable surface area to that of the glass vessel (40 ml water was calculated to cover approx. 63 cm^2 of

the internal surface area). Thus introduction of microplastics at this concentration effectively doubles the surface area available for chemical binding. Each jar was allowed to equilibrate for 24 hours before introduction of the organisms (Lee et al., 2002).

Control treatments consisted of artificial freshwater only (further referred to as 'control'), artificial freshwater with microplastics only (equal to microplastic concentrations in pesticide exposures: 300 000 particles ml^{-1}, further referred to as 'microplastic control'), artificial freshwater with acetone (0.1 %, further referred to as 'acetone control'), and artificial freshwater with both microplastics (300 000 particles ml^{-1}) and acetone (0.1%) (further referred to as 'microplastic and acetone control'). These solutions were made and distributed to glass jars 24 hours prior to introduction of daphnids as per pesticide treatments.

2.5. Acute Toxicity Tests

Following the equilibration period, five neonates (< 24 hours old) were added to each jar. Errors were made in some vessels with 4 neonates added to a vessel (4 vessels overall) or 6 neonates added to a vessel (3 vessels overall). This was taken into account during the data analysis. Jars were completely randomised throughout the exposure to avoid systematic bias. *Daphnia* were observed at 5, 8, 21, 28, 48 and 72 hours. To enable resuspension of any settled particles, each test jar was gently mixed at each observation point by drawing approx. 1-2 ml of exposure media in and out of a glass pipette three times. Aqueous pH was measured in one jar from each concentration at the beginning and the end of the test. The organisms were not fed for the duration of the experiment. Mortality was recorded as per OECD protocol 202 (OECD, 2004). Impaired mobility was also recorded at each time point. This was defined as an individual that was alive, as seen by the clear movement of limbs, but was not able to swim effectively *i.e.* swimming erratically or not swimming effectively in a forward direction, and additionally showing no response to gentle agitation with a glass pipette tip. Sub-lethal behavioural effects are commonly seen in organisms when testing pesticides with a neurotoxic mode of action (Desneux et al., 2007; Haynes, 1988; Sørensen et al., 1995).

2.6. Chemical analysis

Water samples for chemical analysis were taken (1 ml dimethoate, 2 ml deltamethrin) at 0, 24 and 72 hours after preparation of the solutions for deltamethrin treatments and 0 and 72 hours

for dimethoate treatments. Fewer dimethoate measurements were taken than for deltamethrin, as dimethoate was expected to be less complex in terms of chemistry, with concentrations not expected to change over time (Eichelberger and Lichtenberg, 1971; Roast et al., 1999). Samples were spun in 1ml glass tubes (2 tubes per sample) in a centrifuge at approx. centrifugation 6000 G (8000 rpm) for 5 minutes (Eppendorf 24-place Fixed-angle rotor, FA-45-24-11-HS) to remove microplastics and samples were subsequently stored in a fridge at 5°C in the dark prior to analysis. Three replicate samples were taken from a medium and a high nominal concentration for each chemical (0.625 and 5 mg l^{-1} dimethoate, 0.4 and 10 µg l^{-1} deltamethrin) at each of the above specified time points. Chemical analysis was carried out by Wageningen Environmental Research (Alterra), and full details of chemical sampling and analytical procedures are available in the Supplementary Information (SI).

2.7. Data analysis

To determine differences between treatments with and without microplastics at different time points for each chemical, survival frequency data for each chemical were analysed using a Chi-squared (χ^2) test (Microsoft Excel), where treatments without microplastics were the 'expected' and those with microplastics were the 'observed'. Mobility frequency data were analysed using Fisher's exact test (R statistical software) due to a number of zero values (no daphnids swimming normally) which would not be accurately represented using the χ^2. Both tests accounted for any odd numbers where too few or too many neonates had been added initially. Effects on survival and mobility with respect to chemical concentrations and time were evaluated using ANOVA for each endpoint and each chemical, with time points and concentrations considered as factors (R statistical software). A *post-hoc* Tukey HSD test was carried out to determine pairwise differences with time and concentration (R statistical software). Chemical data were analysed using ANOVA with time considered as a factor. A *post-hoc* Tukey HSD test was carried out to determine pairwise differences with time and nominal concentration (R statistical software).

Further analyses of the survival data over time were carried out using a process-based survival model. The model assumes that the toxicant must be first taken up in the organism before it can exert an effect. The kinetics are described with a one-compartment model and the effects is described with the 'stochastic death' model. The model is extensively described in Jager et al. (2006a) and Kooijman and Bedaux (1996). This model is accepted by the OECD (OECD,

2006), where an additional elaborate (mathematical) description can be found with examples of the use of the model. The model links exposure concentrations to a survival probability using three parameters for the whole time-course of the exposure (the No Effect Concentration (NEC): a threshold for toxic effects, the killing rate (k_r): a measure for the toxic potency of the compound, and the elimination rate (k_e) as a kinetic parameter).

Parameter values for dimethoate were calculated using the known (measured) chemical exposure concentrations and the survival data. The parameter values were subsequently compared to independent values obtained from literature for verification. For deltamethrin, the uncertainties related to the actual exposure concentrations prompted a 'reverse modelling' approach. Literature toxicity values for deltamethrin to *D. magna* (Xiu et al., 1989) were used to derive the model parameters, which were subsequently used to fit the model output to the survival data, allowing back-calculation of actual exposure concentrations (further details on this approach are available in the SI). The benefits of including the modelling are threefold: 1) to validate the results of the traditional statistical analysis, 2) to calculate the actual concentrations of pesticides that the *Daphnia* are exposed to and 3) to determine toxicity effects over time, allowing for extrapolation of toxicity estimates beyond the timeframe of the experiments. Together, these benefits allowed us to better understand the dynamics of toxicity within the experiment.

3. Results

3.1. Daphnia survival

Daphnia survival in the controls without microplastics or chemicals, and in the acetone controls, was 100%. This high control survival validates the criteria of the toxicity test according to OECD guidelines for *Daphnia magna* acute toxicity testing (OECD, 2004). Microplastics alone did not affect survival over the 72 hour test period with only one mortality in the microplastic control treatment (5%) after the 72 hour exposure period and 100% survival in the microplastics and acetone control treatments. While it may be the case that some particles could have aggregated and therefore were not within an edible size range for *D. magna*, without the use of a microscope, microplastics were clearly visible within the guts of daphnids as a white mass indicating that ingestion did occur during the exposure.

There was a significant effect of pesticide exposure concentration on survival ($p < 0.01$ for both pesticides, ANOVA). There were also a significant effect of exposure time on survival (p

< 0.01 for both pesticides, ANOVA) and a significant interaction between concentration and time also occurred ($p < 0.01$ for both pesticides, ANOVA). Over the 72 h exposure, significant effects were seen on survival at exposure concentrations above 1.25 mg l^{-1} for dimethoate ($p < 0.01$, ANOVA + Tukey HSD) and above 0.4 µg l^{-1} for deltamethrin ($p < 0.05$, ANOVA + Tukey HSD). When considering time, significant effects on survival were seen from 48 hours in dimethoate treatments above 2.5 mg l^{-1} ($p < 0.01$, ANOVA + Tukey HSD, Table 1a) and from 28 hours in deltamethrin treatments above 2 µg l^{-1} ($p < 0.01$, ANOVA + Tukey HSD, Table 2a). For both pesticides there was no significant difference in the survival of organisms based on the presence or absence of microplastics ($p > 0.05$ at every time point, χ^2) To give a visual representation of this similarity, the survival and mobility probability was calculated and the deviance between treatments with and without microplastics depicted (Figs. 1a and 2a). Deviance was calculated as the difference in survival (or mobility) probabilities for treatments without MPs (– MP) vs. those with MPs (+ MP) at given concentrations.

3.2. Daphnid mobility

There were also concentration-dependent effects on daphnid mobility. There was a significant effect of pesticide exposure concentration on mobility ($p < 0.01$ for both pesticides, ANOVA). There were also a significant effect of exposure time on mobility for both chemicals ($p < 0.01$ for both pesticides, ANOVA) and a significant interaction between concentration and time also occurred for both chemicals (ANOVA, $p < 0.01$ for both chemicals). Over the 72 h exposure, significant mobility impairment was observed in *Daphnia* exposed to dimethoate at concentrations of 1.25 mg l^{-1} and above ($p < 0.01$, ANOVA + Tukey HSD). Similarly, *Daphnia* exposed to 0.08 µg l^{-1} deltamethrin and above suffered significant mobility impairment ($p < 0.05$, ANOVA + Tukey HSD). When considering time, significant effects on mobility were seen from 21 hours for dimethoate at 5 mg l^{-1} ($p < 0.01$, ANOVA + Tukey HSD, Table 1b) and from 5 hours for deltamethrin at 10 µg l^{-1} ($p < 0.01$, ANOVA + Tukey HSD, Table 2b). The presence of microplastics resulted in no significant difference in the number of daphnids suffering impaired mobility for either chemical at any time point ($p > 0.05$, Fisher's exact test). As for survival, plots for deviance were created to give a visual representation of this similarity using deviance in probability of normal mobility of treatments with vs. without microplastics (Figs 1b and 2b). Effects on mobility were seen at earlier time points than effects on survival, as would be expected given that sublethal behavioural effects are a precursor to mortality.

Table 1. Survival probabilities (Table 1a) and probabilities of normal mobility (Table 1b) for *D. magna* exposed to dimethoate at each concentration and time point, calculated by dividing the remaining surviving neonates by the original 20 to give a probability between 0-1.

Table 1a)

Dimethoate exposure concentration (mg l^{-1})		Time (h) 0	5	8	21	28	48	72
0	**Without MP**	1	1	1	1	1	1	1
0	**With MP**	1	1	1	1	1	1	1
0.156	**Without MP**	1	1	1	1	1	1	1
0.156	**With MP**	1	1	1	1	1	0.9	0.8
0.313	**Without MP**	1	1	1	1	1	1	1
0.313	**With MP**	1	1	1	1	1	0.6	0.5
0.625	**Without MP**	1	1	1	1	1	1	1
0.625	**With MP**	1	1	1	1	1	0.9	0.6
1.25	**Without MP**	1	1	1	1	1	1	0.6
1.25	**With MP**	1	1	1	1	1	0.9	0.7
2.5	**Without MP**	1	1	1	0.8	0.8	0.4	0
2.5	**With MP**	1	1	1	1	1	0.6	0
5	**Without MP**	1	1	1	0.7	0.7	0.2	0.1
5	**With MP**	1	1	1	0.9	0.8	0.2	0

Table 1b)

Dimethoate exposure concentration (mg l^{-1})		Time (h) 0	5	8	21	28	48	72
0	**Without MP**	1	1	1	1	1	1	1
0	**With MP**	1	1	1	1	1	1	1
0.156	**Without MP**	1	1	1	1	1	1	1
0.156	**With MP**	1	1	1	1	1	1	0.8
0.313	**Without MP**	1	1	1	1	1	1	1
0.313	**With MP**	1	1	1	1	1	0.8	0.4
0.625	**Without MP**	1	1	1	1	1	1	1
0.625	**With MP**	1	1	1	1	1	0.9	0.5
1.25	**Without MP**	1	1	1	1	1	0.9	0.4
1.25	**With MP**	1	1	1	1	1	0.7	0.6
2.5	**Without MP**	1	1	1	1	0.5	0	0
2.5	**With MP**	1	1	1	0.9	0.7	0.2	0
5	**Without MP**	1	1	1	0.7	0.3	0	0
5	**With MP**	1	1	1	0.4	0.4	0	0

Table 2. Survival probabilities (Table 2a) and probabilities of normal mobility (Table 2b) for *D. magna* exposed to deltamethrin at each concentration and time point, calculated by dividing the remaining surviving neonates by the original 20 to give a probability between 0-1.

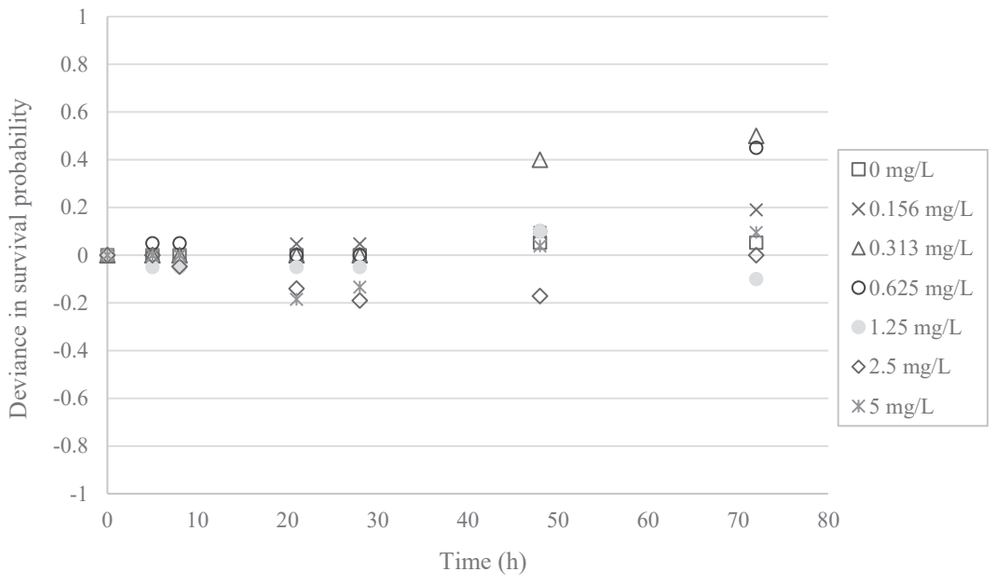
Table 2a)

Deltamethrin exposure concentration (µg l^{-1})		Time (h) 0	5	8	21	28	48	72
0	**Without MP**	1	1	1	1	1	1	1
0	**With MP**	1	1	1	1	1	0.9	1
0.016	**Without MP**	1	1	1	1	1	1	1
0.016	**With MP**	1	1	1	1	1	1	1
0.08	**Without MP**	1	1	1	1	1	1	0
0.08	**With MP**	1	1	1	1	1	0.9	0
0.4	**Without MP**	1	1	1	0.9	1	0.9	0
0.4	**With MP**	1	1	1	1	0.9	0.7	0
2	**Without MP**	1	1	1	0.9	0.7	0.5	0
2	**With MP**	1	1	1	1	0.7	0.6	0
5	**Without MP**	1	1	1	0.7	0.7	0.2	0
5	**With MP**	1	1	1	0.8	0.8	0.5	0
10	**Without MP**	1	1	1	1	0.7	0.3	0
10	**With MP**	1	1	1	1	0.6	0.2	0

Table 2b)

Deltamethrin exposure concentration (µg l^{-1})		Time (h) 0	5	8	21	28	48	72
0	**Without MP**	1	1	1	1	1	1	1
0	**With MP**	1	1	1	1	1	0.9	1
0.016	**Without MP**	1	1	1	1	1	1	1
0.016	**With MP**	1	1	1	1	1	1	1
0.08	**Without MP**	1	1	1	0.9	1	0.7	0
0.08	**With MP**	1	1	1	1	1	0.6	0
0.4	**Without MP**	1	1	1	0.7	0.4	0.2	0
0.4	**With MP**	1	1	1	0.7	0.3	0	0
2	**Without MP**	1	0.9	0.8	0.1	0.1	0	0
2	**With MP**	1	1	0.8	0.1	0	0	0
5	**Without MP**	1	0.9	0.7	0.1	0.1	0	0
5	**With MP**	1	1	0.6	0.1	0.2	0.1	0
10	**Without MP**	1	0.4	0.6	0.1	0	0	0
10	**With MP**	1	0.4	0.6	0.2	0	0	0

1a



1b

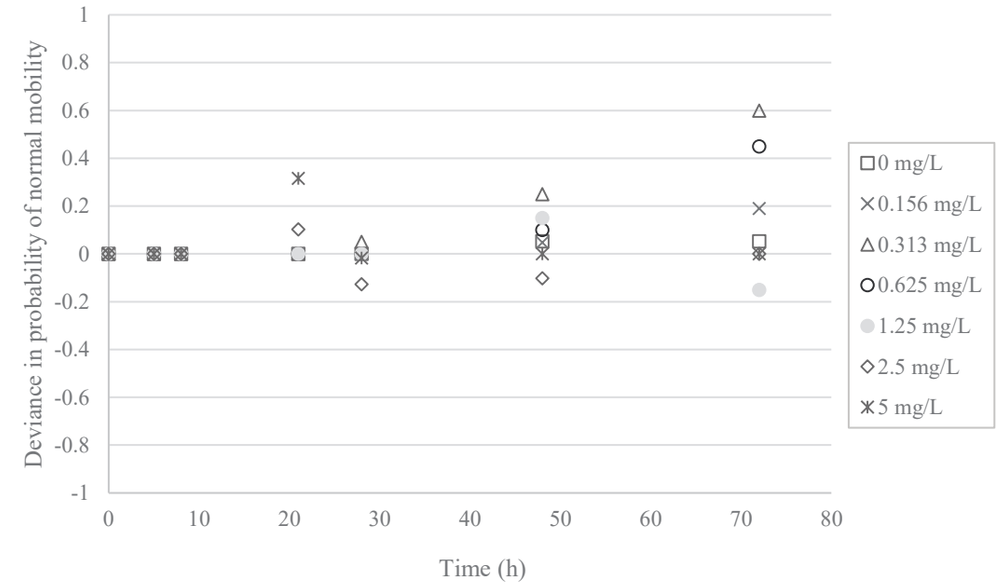
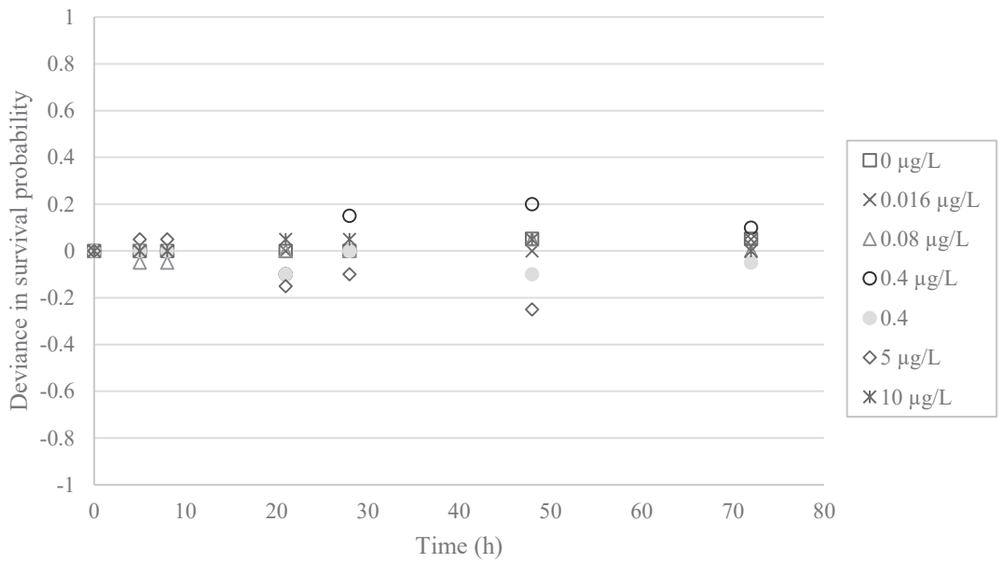


Fig. 1. Data for dimethoate showing 1a) a comparison of survival probabilities (the deviance in survival probability based on a ratio of survival probability without microplastics and with microplastics) and 1b) a comparison of normal mobility probabilities (calculated as for 1a). Deviations from 0 indicate the extent of the difference when microplastics were present. The closer to 0, the more similar the data. Full survival and mobility probability values for dimethoate are presented in Tables 1a and 1b respectively.

2a



2b

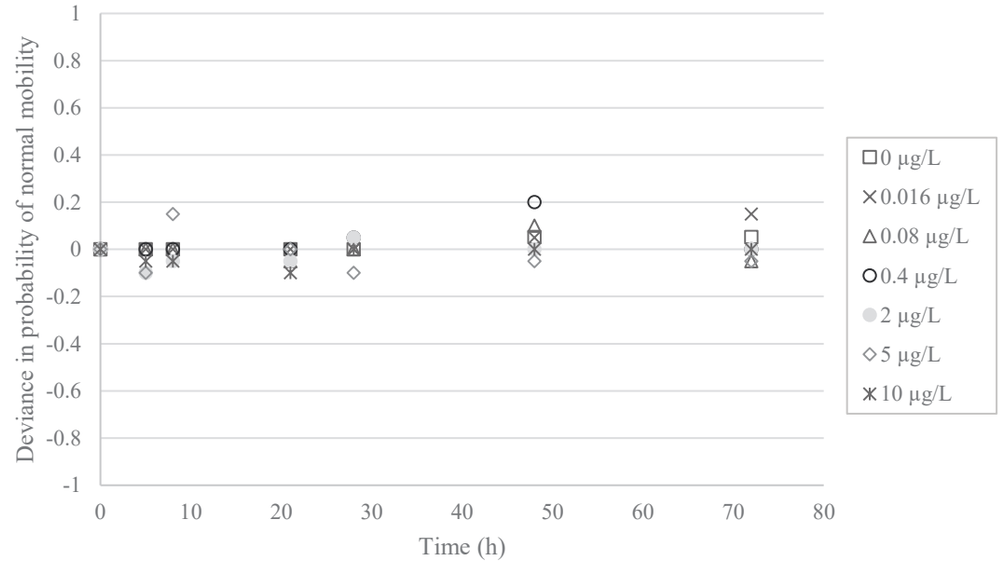


Fig. 2. Data for deltamethrin showing 2a) a comparison of survival probabilities (the deviance in survival probability based on a ratio of survival probability without microplastics and with microplastics) 2b) a comparison of normal mobility probabilities (calculated as for 2a). Deviations from 0 indicate the extent of the difference when microplastics were present. The closer to 0, the more similar the data. Full survival and mobility probability values for deltamethrin are presented in Tables 2a and 2b respectively.

3.3. Chemical concentrations

The pH remained consistent throughout the test with a mean pH of 7.81 (± 0.17 SD) across treatments at 0 hrs and 7.9 (± 0.05 SD) at 72 hours.

All measured dimethoate concentrations were lower than the nominal concentrations, ranging from (average) 59-63% of nominal values, although this difference was not significant ($p > 0.05$, t-test, Table S1). Measured concentrations of dimethoate did not vary significantly over time ($p > 0.05$, ANOVA) and there was no effect of microplastics on the measured concentrations of dimethoate ($p > 0.05$, ANOVA) (Figs. 3a and 3b). There was no significant effect of microplastics on concentration over time (interaction $p > 0.05$, ANOVA).

There was a significant difference between nominal and measured deltamethrin concentrations ($p < 0.01$, t-test), with average measured concentrations ranging from 3.7-20.5% of the nominal concentrations (Table S2). Due to an apparent difference in trend between the low and high nominal concentrations measured (Figs. 4a and 4b), these were analysed separately to tease apart concentration-dependent effects. At the low nominal concentration (0.4 $\mu g\ l^{-1}$), there was no effect of microplastics or time on the measured concentrations (both $p > 0.05$, ANOVA), nor an interaction of time and microplastics ($p > 0.05$, ANOVA). At the highest nominal concentration (10 $\mu g\ l^{-1}$), both microplastics and time significantly influenced the measured concentrations, with concentrations lower when microplastics were present (both microplastics and time $p < 0.01$, ANOVA), and with an initial significant decrease in concentration up to 24 hours (0-24 h, $p < 0.01$, ANOVA + Tukey HSD, 24-72 h, $p > 0.05$, ANOVA + Tukey HSD). There was no significant effect of microplastics on concentration over time (interaction $p > 0.05$, ANOVA).

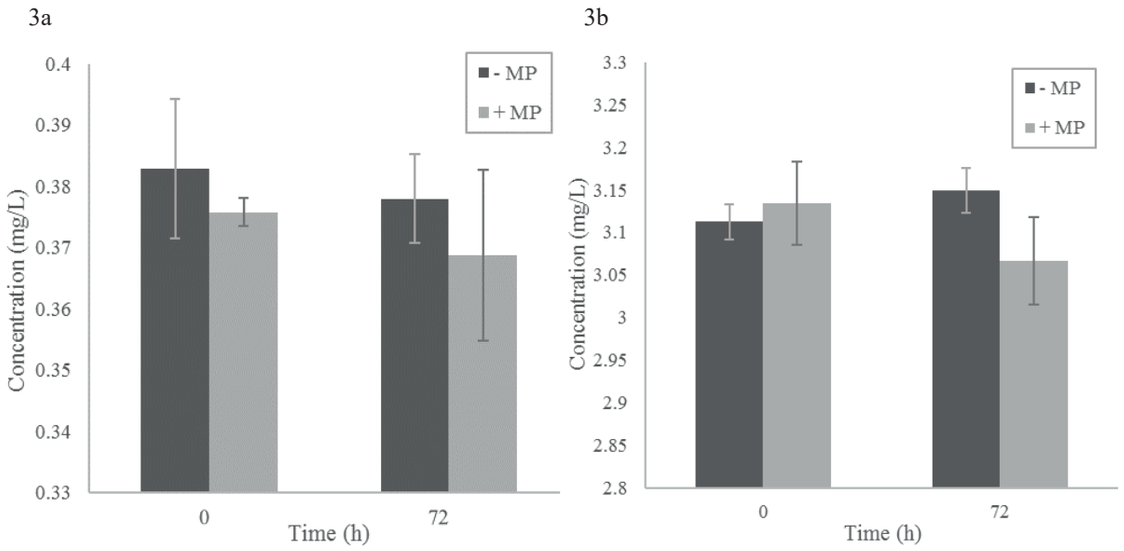


Fig. 3. Average measured concentrations based on three replicate samples of dimethoate (± SD) at different time points taken from treatments with nominal concentrations (a) 0.625 mg l^{-1} and (b) 5 mg l^{-1}, with or without microplastics, at each time point. '- MP' = no microplastics, '+ MP' = with microplastics.

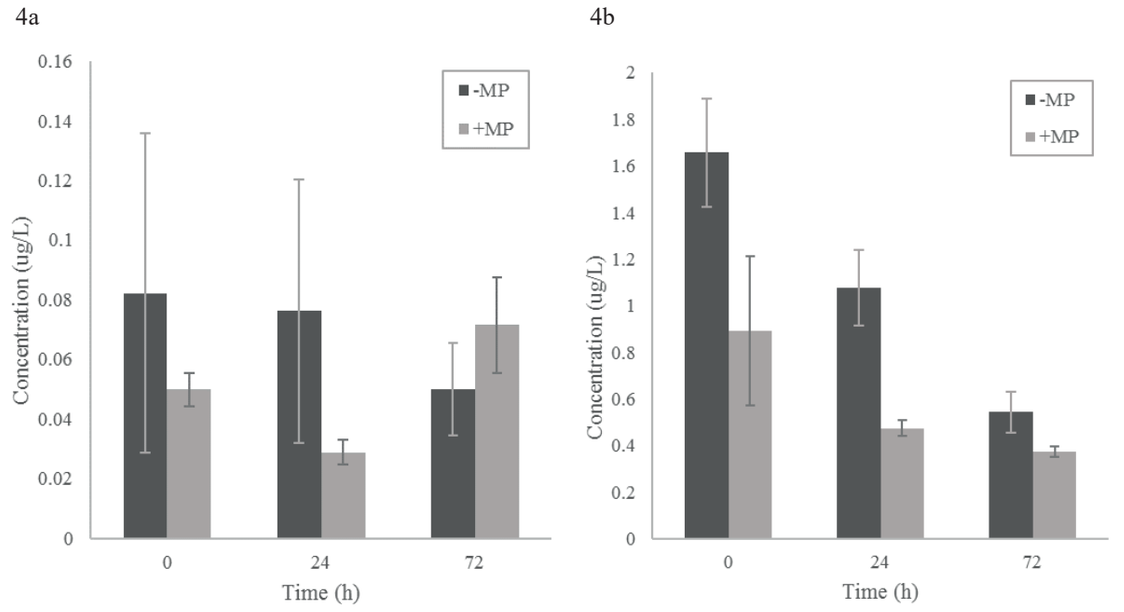


Fig. 4. Average measured concentrations based on three replicate samples of deltamethrin (± SD) at different time points taken from treatments with nominal concentrations (a) 0.4 µg l^{-1} and (b) 10 µg l^{-1} b, with or without microplastics, at each time point. '- MP' = no microplastics, '+ MP' = with microplastics.

3.4. Model analysis

Fitting of separate stochastic death models for both dimethoate and deltamethrin gave an estimation of toxicity over time at the experimental exposure concentrations and provided a consistent fit with the survival data (Figs. S2 and S3). For dimethoate, the model-derived LC_{50} was 0.5 mg l^{-1} (the full range of model-derived LC_x values for dimethoate available in Table S6). For deltamethrin, the model-derived LC_{50} was 0.023 µg l^{-1} (the full range of model-derived LC_x values for deltamethrin are available in Table S7). For both pesticides, the model shows no difference in pesticide exposure, or survival, with or without microplastics. For deltamethrin, using the reverse modelling approach, the survival data were used to determine the actual exposure concentrations as an indirect and complementary assessment of the measured concentrations (Table 3).

Table 3. Nominal concentration range of deltamethrin compared to modelled exposure concentrations and measured concentrations.

Nominal Concentration (µg l^{-1})	Nominal Concentration (nM)	Modelled Concentration (µg l^{-1})	Modelled Concentration (nM)	Measured Concentration (µg l^{-1})
0.016	0.03	0.012	0.024	-
0.08	0.16	0.03	0.06	-
0.4	0.79	0.04	0.079	0.05
2	3.96	0.08	0.16	-
5	9.9	0.08	0.16	-
10	19.79	0.09	0.18	0.40

The reverse modelling to predict actual exposure concentrations indicated that the concentrations in the three highest test treatments are more or less equal. This is likely governed by the solubility limit, which would therefore be around 0.08-0.09 µg l^{-1} (close to the reported value of 0.2 µg l^{-1} (Mestres and Mestres, 1992; Pesticide Properties Database, 2017a). The reported 48 h LC_{50} taken from literature that informed the parameters used for this model estimation was at the lower end of the scale: 0.038 µg l^{-1} (Xiu et al., 1989), compared to 0.32-0.63 µg l^{-1} reported by (Toumi et al., 2013), although is comparable to that reported in other studies (0.05-0.6 µg l^{-1} reported by (Day and Maguire, 1990). With higher input values the calculated exposure concentrations may have been higher.

4. Discussion

4.1. Biological effects

Although microplastics are commonly implicated in causing physiological damage to organisms, leading to reduced fitness and mortality (Lee et al., 2013; Rehse et al., 2016; Wright et al., 2013a), no microplastic-specific effects on mobility or survival were seen in this acute test, despite the high concentration of microplastics used and visual confirmation of ingestion. This result is in accordance with a number of other studies where high concentrations of microplastics were shown to cause no observable detrimental effects (Hämer et al., 2014; Kaposi et al., 2014; Weber et al., 2018b). Although other acute studies have measured subtle effects of exposure to microplastics that may have occurred, for example immune responses, gut blockage, reduced assimilation efficiency or reduced scope for growth (Blarer and Burkhardt-Holm, 2016; Cole et al., 2015; Jeong et al., 2016; Lo and Chan, 2018), these were beyond the scope of this study which was not planned to determine the effects of microplastics alone, but to determine whether the presence of microplastics influenced the toxic effects of pesticides.

Contrary to the hypothesis that microplastics would lead to a reduction in toxic effect of the high log Kow pesticide deltamethrin, the results showed no alteration in the acute toxicity of either deltamethrin or dimethoate to *D. magna*, regardless of the chemical binding capacity (log Kow) (Figs. 1 and 2, Tables 1 and 2). Mortality and mobility impairment increased with concentration and time for both pesticides, as expected, however the concentrations at which detrimental effects occurred were not influenced by the presence of microplastics. This is also highlighted by the results of the stochastic death modelling.

4.2. Linking biological effects to chemical exposure

The measured concentrations for deltamethrin were significantly lower than expected across all treatments, on average between 3.7-20.5 % the nominal concentration, depending on the time the sample was taken and the presence of microplastics (Fig. 3). Measured concentrations were highly variable, especially at the lower measured concentrations when microplastics were present (Fig. 4a). Additional replicate samples would have helped to reduce this variability and may have helped to clarify whether the lack of significance was simply due to high variability. However, regardless of the significant differences found in measured deltamethrin

concentrations between treatments with and without microplastics at higher concentrations (Fig. 4b), no differences in toxicity were observed. This highlights that the chemical dynamics within the system were complex and that while some binding of pesticides to microplastics may have occurred, this did not reduce the bioavailability of the two pesticides enough to lower the resulting observed toxicity. As predicted, there was no significant difference in water concentration with or without microplastics for dimethoate, supporting the lack of difference in the survival and mobility data, and no significant change in concentrations over time (Fig. 3). This difference between deltamethrin and dimethoate highlights that hydrophobicity of chemicals can influence binding and removal from solution, influencing different chemicals in different ways, however toxicity is more complex to predict.

Due to the high hydrophobicity of deltamethrin, it is likely that this pesticide bound strongly to both the glass vessel and the microplastic particles (where present) (Lee et al., 2002; Sethi et al., 2014; Wheelock et al., 2005). To overcome this, we introduced a 24 h equilibrium period following the suggestion made by Lee et al. (2002). Nonetheless it turned out extremely difficult to make accurate quantifications of the deltamethrin concentrations in water, as deltamethrin is also likely bind to organic matter including the *Daphnia* and any associated organic detritus or excreta. This means that, despite the 24 h equilibration phase, the equilibrium likely shifted when the *Daphnia* were introduced to the solution, highlighted by the significant reduction in concentration within the aqueous solution within the first 24 hours. This is a highly dynamic system and the equilibrium is likely to continue to shift over time leading the chemical to be associated with different substrates at different times. This highlights the complexity of working with deltamethrin, with binding, availability and ease of chemical extraction dependent on substrates available and methods used.

Due to the discrepancy between measured and nominal concentrations for deltamethrin, we were not able to directly relate toxicity to nominal or measured chemical concentrations. It was for these reasons that we carried out the reverse modelling approach to determine the likely exposure concentrations the *Daphnia* were exposed to (Table 3) and thus enable us to determine the toxicity of deltamethrin (SI). The model showed that, probably as a result of the limit of solubility of the hydrophobic insecticide, the top three concentrations of deltamethrin (nominal concentrations 2, 5, and 10 $\mu g\ l^{-1}$) were in fact likely to have been almost identical at 0.08-0.09 $\mu g\ l^{-1}$ (Table 3). This was reflected in the survival and mobility matrices showing survival and mobility to be comparable across the top three concentrations (comparing top three concentrations across survival and mobility, all $p > 0.05$ ANOVA + Tukey HSD, Table

2). This highest calculated exposure concentration was below the expected lower limit of solubility (0.2 µg l^{-1} at 25°C). This could be due to the combined effects of a lower temperature than stated for maximum solubility (experiments were run at 20°C ± 1°C) and additional dissolved constituents in the Elendt artificial freshwater, both of which may have led to a decreased capacity for dissolution.

Although the highest concentrations of deltamethrin used in this study were above solubility, the actual value for solubility is uncertain, reported between 0.2-2 µg l^{-1} (Mestres and Mestres, 1992). EC_{50} values for deltamethrin for effects on mortality and immobilisation in *D. magna* reported in the literature are highly variable, ranging from 0.11 to 9.4 µg l^{-1} at 24 h and 0.03 to 0.63 µg l^{-1} at 48 h (Toumi et al., 2013; Xiu et al., 1989). The highest of these values, particularly for the 24 h exposure time, hence are well above stated solubility. In this study, the modelled 96 h LC_{50} of 0.023 µg l^{-1} is in the same order of magnitude as the literature value of 0.01 µg l^{-1} calculated by Xiu et al. (1989), although it should be noted that their calculation was based on nominal concentrations. Many studies focus solely on nominal concentrations, not taking into account solubility or binding issues, while studies that do seek to determine concentrations find measured concentrations to be vastly reduced from nominal values (Lee et al., 2002; Toumi et al., 2013; Wheelock et al., 2005).

The modelling allowed us to compare the toxicity observed in this study to literature data (SI) and enabled us to develop a better understanding of the biological effects seen under given chemical and microplastics exposures. For dimethoate, measured concentrations were much closer to stated nominal concentrations, and were consistent over time. Model estimations for toxicity of dimethoate in this study based on the measured chemical data showed exposures to be comparable with or without microplastics, with our LC_{50} results shown to be comparable to literature values (SI).

4.3. Binding of pesticides to microplastics

Different polymers have different affinities for chemical binding and therefore may have differing propensities for altering the toxicity of associated chemicals. For example, it has been reported that polyethylene and polypropylene will have greater affinities for chemical sorption than polyvinyl chloride (PVC) or polyethylene terephthalate (PET) (Rochman et al., 2013b). Polystyrene has been suggested as having a lower affinity for hydrophobic chemical sorption than polyethylene, but higher than PVC (Wang and Wang, 2018). It is nonetheless recognised

that polystyrene will associate with hydrophobic organic chemicals within the environment (Liu et al., 2015; Rochman et al., 2013d). The concentration of polystyrene particles used in this experiment (300 000 particles ml^{-1}) is far above the concentrations that will likely be found within the freshwater environment (see Horton et al. (2017b) for an overview of freshwater microplastic studies), although this exposure level is within the range of other experimental studies using microplastics (Lu et al., 2016; Ogonowski et al., 2016; Rehse et al., 2016; Setälä et al., 2014). This study was therefore intended to give a representation of the possible effects of interactions between microplastics, pesticides and freshwater organisms in a scenario where microplastics were highly abundant.

The presence of microplastics would have provided an increased surface area available for chemical binding (in this instance the surface area of the microplastics was calculated to be approximately equivalent to that of the vessel, effectively doubling the surface area). Therefore a lower concentration of deltamethrin would have been expected in the water when microplastics were present. The chemical measurement results confirm this effect, as at the highest exposure concentration of deltamethrin (nominal concentration of 10 µg/l), water concentrations were significantly lower when microplastics were present (Fig. 4b). This implies that deltamethrin was binding to microplastics (inferred by a reduced concentration in water when compared to an equivalent nominal concentration without microplastics). However, it is important to note that despite the difference with and without microplastics at the highest concentration of deltamethrin (nominal concentration 10 µg l^{-1}), the reduced concentration in the presence of microplastics was not observed at the lower concentration measured (nominal concentration 0.4 µg l^{-1}) (Fig. 4a). In the higher nominal exposure levels (10 µg l^{-1}), the decline in measured concentration continues after the 24 h equilibration period highlighting the complex chemical dynamics within the solution, with the introduction of daphnia likely to alter the equilibrium. Questions remain surrounding the dynamics and kinetics of chemical behaviour and toxicity in relation to the presence of microplastics. However, as there were no significant effects on survival and mobility between microplastic and non-microplastic treatments in this study, these complex dynamics do not appear to affect the overall bioavailability, and as a result, acute toxicity of the chemicals.

4.5. Outlook

If effects are to be seen with respect to chemicals in association with microplastics, especially their facilitation of chemical uptake and toxicity, it is most likely that these would be seen under controlled laboratory conditions where uncontaminated organisms are exposed to contaminated plastics (of a size that enables ingestion), as opposed to in the environment where organisms will already have been exposed to a variety of different chemicals (Koelmans et al., 2016). This study was designed to enable optimum chemical binding and ingestion of microplastics by *D. magna*. Given the high concentration of microplastics in this study and, thus, the high surface area available for binding, an alteration in the bioavailability and toxicity of hydrophobic deltamethrin (high log Kow) would have been expected, whereas dimethoate (low log Kow) would be expected to be consistently bioavailable and toxic regardless of the presence of microplastics (Cole et al., 2011; Teuten et al., 2009). In contrast, our results show that there was no effect of microplastics on the response of daphnids to either of the two pesticides, despite the very different chemical characteristics. The vector effects, or so-called 'Trojan Horse' effects, as ascribed to microplastics (Rochman et al., 2014; Rochman et al., 2013d) were not observed. It is therefore unlikely that microplastics will exert short-term effects on pesticide toxicity under real field conditions where sediment and organic matter would compete with microplastics for binding of chemicals. Additionally, in areas highly polluted with pesticides or other organic chemicals, the presence of microplastics is unlikely to alter the availability of these pollutants (Tanaka et al., 2018). In terms of chemical toxicity associated with microplastics, it is feasible that plasticisers will pose a greater chemical risk to organisms than sorbed hydrophobic chemicals (Devriese et al., 2017; Lohmann, 2017). Although polymer, particle and chemical-specific, these data are a valuable contribution to the wider understanding of microplastic and chemical associations, and the complexities underlying these mechanisms.

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CHAPTER 5

SUPPLEMENTARY INFORMATION

S1. Area and mass calculations

S1.1. Surface area calculations

Particles were calculated using TEM as being 1.2 µm ± 0.2 µm in diameter (fig S1). Surface area was therefore calculated for particles of 1 µm and 1.4 µm to account for variation, using the equation:

$A = 4\pi r^2$ (equation 1)

Calculated surface area ranged from 3.14 μm^2 for a 1 µm particle and 6.15 μm^2 for a 1.4 µm particle (median 1.2 µm ± 0.2 µm). Given a concentration of 300 000 particles ml^{-1}, the number in 40 ml solution was approximately 12 000 000. This therefore gave a total particle surface area per vessel of between 37.7 cm and 73.9 cm.

The surface area of the inside of the vessel was calculated to be approximately 62.8 cm^2 based on a depth of 3.8 cm and a diameter of 4.2 cm when filled with 40 ml water.

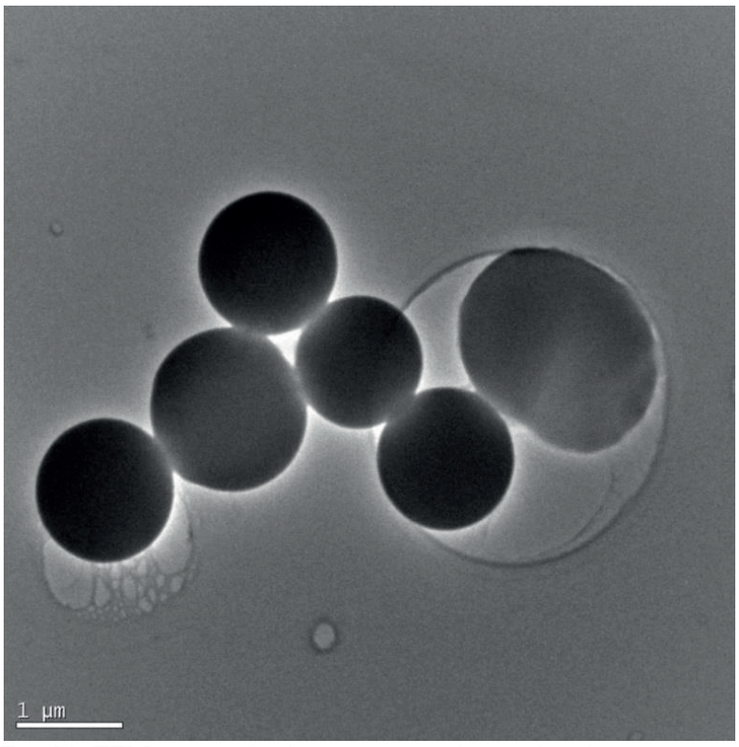


Fig. S1. TEM image of polystyrene particles used in the exposures.

S1.2. Particle mass calculations

Particle mass was calculated by taking the known particle density: 1.06 g cm^{-3}, and the mean particle radius: 0.6 µm (0.00006 cm). The volume of an individual sphere was calculated using the equation:

$V=4/3\ \pi r^3$ *(equation 2)*

This gave a particle volume of 9.05 x 10^{-13} cm^3. Volume was then multiplied by density to give the mass of one particle: 9.59 x 10^{-13} g (9.59 x 10^{-7} µg). This could then be multiplied by 300 000 to give the mass of particles per ml: 2.88 x 10^{-7} g ml^{-1} (0.29 µg ml^{-1}) and then by 1000 to give the mass of particles per l: 0.00029 g l^{-1} (287.7 µg l^{-1}).

S2. Chemical analysis methods

For the dimethoate treatments, 1 ml samples were taken from three replicate vessels of two different nominal concentrations (5 mg l^{-1} and 0.625 mg l^{-1}) at 0 and 72 hours and the microplastic treatments centrifuged as before. From the centrifuged microplastic samples, 800 µl was carefully pipetted into a glass vial to avoid resuspending the particles and 400 µl methanol added. The non-microplastic samples were not centrifuged and 500 µl methanol was added to the 1 ml sample. Vials were tightly sealed with a cap (phenolic cap with aluminium liner) and were then shaken well to mix.

For the deltamethrin treatments, 2 ml samples were taken from three replicate vessels of two different nominal concentrations (10 µg l^{-1} and 0.04 µg l^{-1}) at 0, 24 and 72 hours (based on times of daphnia exposure). Following removal, the microplastic samples were immediately spun in 1ml glass tubes (2 tubes per sample) in a centrifuge at approx. 6000 G (8000 rpm) for 5 minutes (Eppendorf 24-place Fixed-angle rotor, FA-45-24-11-HS) and the 1.6 ml (800 µl per tube) supernatant carefully pipetted off to avoid resuspending the particles. This was transferred to a glass vial and 1.6 ml hexane added. The non-microplastic samples were not centrifuged and 2 ml hexane was added to the 2 ml sample. The microplastic and non-microplastics samples were then treated the same by shaking the sample with the hexane vigorously for 1 minute in a glass vial tightly sealed with aluminium foil and parafilm and then pipetting 1.2 ml of the hexane fraction into a 2ml brown glass vial (Sigma Aldrich). Vials were tightly sealed with a cap (phenolic cap with aluminium liner, Sigma Aldrich).

All chemical samples were analysed at Wageningen Environmental Research (Alterra). The analytical method was developed at the laboratory of the Environmental Risk Assessment team.

Dimethoate samples were diluted 100 times with acetonitrile-ultrapure water by using a Dilutor Hamilton 600 series. The diluted samples were analysed using an Agilent LC-MS×MS suite (Agilent 6460 Triple Quad LC/MS) equipped with autosampler (Agilent G1329B), pump (Agilent G1311B (Quat. pump)), an ESI (+Agilent Jet Stream) source and a column thermostat (Agilent G1316A). The separation was performed in reverse phase LC (Column: Agilent Zorbax Eclipse XDB C18; 4.6 mm x 150 mm, 5 micron) under gradient elution of Eluents C (Milli-Q water (Advantage A10) + 0.1 % v/v formic acid) and Eluent D (Acetonitrile + 0.1 % formic acid). The initial composition of the mobile phase (40%:60%, C:D) was first held for 2 mins, then changed in 1 min to 20%:80% (C:D) (between 2 and 3 minutes run time), held for 3 minutes (between 3 and 6 minutes run time), changed back to the initial composition over 1 minute (between 6 and 7 minutes) and held there 1 more minute (between 7 and 8 minutes). The flow rate and column temperature were fixed at 0.7 $mL.min^{-1}$ and 35°C, respectively. Dimethoate retention time was ca. 2.5 minutes and was detected by monitoring the 230 m/z – 198.9 m/z transition (quantifier), qualified with additional peaks at m/z = 171 and 125. Injected samples were quantified by peak area using the calibration curve constructed from calibration standards included in the same sample sequence.

Deltamethrin was measured in the hexane extract by using an Agilent 6890 gas chromatograph equipped with an electron capture detector (ECD). Three microliters of the extract was injected via split injection and analysed in a wall-coated open tubular (WCOT) fused silica column (Varian CP Sil5) using He gas as the mobile phase. The oven temperature was programmed so that the initial temperature of 50°C was held for 7 minutes after which, the temperature was ramped at a rate of 50°C min^{-1} to a final temperature of 300°C minutes and held for 15:30 minutes. Retention time for deltamethrin was approximately 25.3 minutes. Injected samples were quantified by peak area using the calibration curve constructed from calibration standards included in the same sample sequence.

Table S1. Nominal and average measured concentrations (three replicate samples) for dimethoate treatments

Nominal concentration (mg l^{-1})	Microplastic treatment	Time point	Average measured concentration (mg l^{-1})	Standard deviation
0.625	NO	0	0.383	0.011
0.625	NO	72	0.378	0.007
0.625	YES	0	0.376	0.002
0.625	YES	72	0.369	0.014
5	NO	0	3.112	0.021
5	NO	72	3.149	0.027
5	YES	0	3.134	0.049
5	YES	72	3.067	0.051

Table S2. Nominal and average measured concentrations (three replicate samples) for deltamethrin treatments

Nominal concentration (µg l^{-1})	Microplastic treatment	Time point	Average measured concentration (µg l^{-1})	Standard deviation
0.4	NO	0	0.082	0.054
0.4	NO	24	0.076	0.044
0.4	NO	72	0.050	0.015
0.4	YES	0	0.050	0.006
0.4	YES	24	0.029	0.004
0.4	YES	72	0.072	0.016
10	NO	0	1.657	0.234
10	NO	24	1.077	0.161
10	NO	72	0.544	0.089
10	YES	0	0.892	0.322
10	YES	24	0.475	0.035
10	YES	72	0.375	0.021

S3. DEB modelling methods

S3.1. Modelling approach

The Stochastic Death model was used to model the data. This model is extensively described in the original paper by Kooijman and Bedaux (1996) and is accepted by the OECD (OECD, 2006). In addition, see Jager et al. (2011) for an extensive review on the different survival models.

The model needs three parameters to describe the whole time course of toxic effects:

1) No Effect Concentration (NEC): a toxicological threshold for effects
2) Killing rate (k_r): a measure for the toxicity of the compound
3) Elimination rate (k_e): a kinetic parameter determining the kinetics of the compound

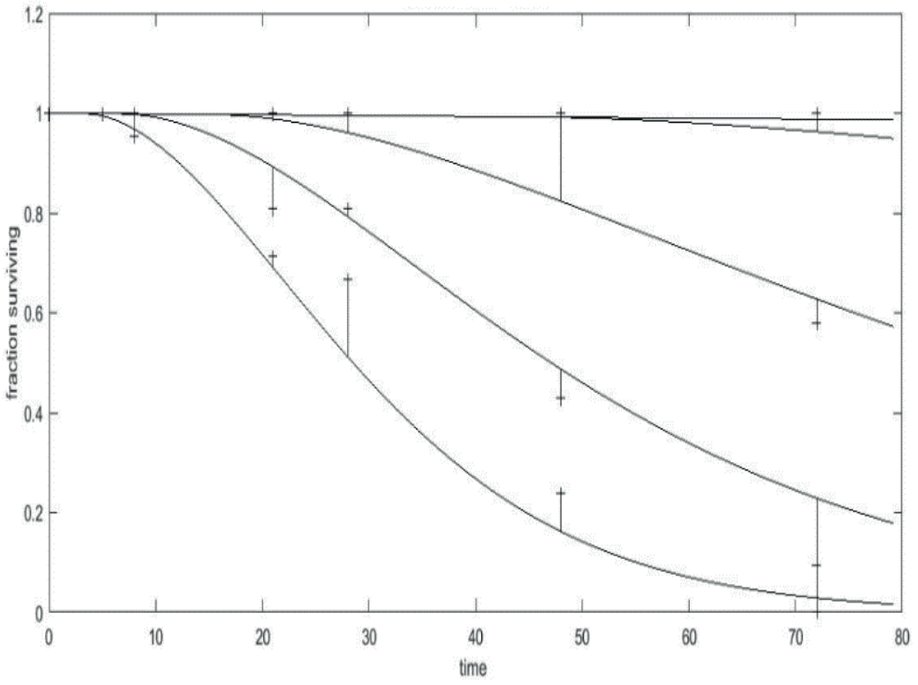
There is an additional parameter (the blank killing rate (BKR)) to take control mortality into account. The NEC is the most important parameter as this reflects the inherent sensitivity of the species for a toxicant. Usually this parameter is also the parameter value with the smallest confidence interval.

Parameter values can be estimated from the raw data of a survival experiment (e.g. Hesketh et al. (2016)), given multiple points in time, as the approach is basically a TK-TD approach. The model can also be used, if the parameter values are known, to back-estimate the exposure concentrations if the survival probabilities are taken from the experiments.

S3.1.1. Dimethoate

Actual concentrations were measured for two nominal concentrations (5 and 0.625 mg/L nominal) at the start of the exposure and at the end of the exposure (24 hrs and 96 hrs after preparing the exposure solutions). Concentrations were stable over the measurement period and there is a constant fraction of the nominal concentrations for the two measured concentrations (0.625 and 5 mg l^{-1}), this fraction equals 61% of the nominal concentrations both for treatments with and without microplastics. The exposure concentrations calculated based on measured values therefore gave a range of 0, 0.08, 0.15, 0.3, 0.6, 1.2, 2.4 mg l^{-1}. There appears to be no effect of the microplastics on the actual concentrations. This was the starting point for the parameter estimates. The results of the parameter estimates are summarised in Table S3 (all expressed in µ moles).

a



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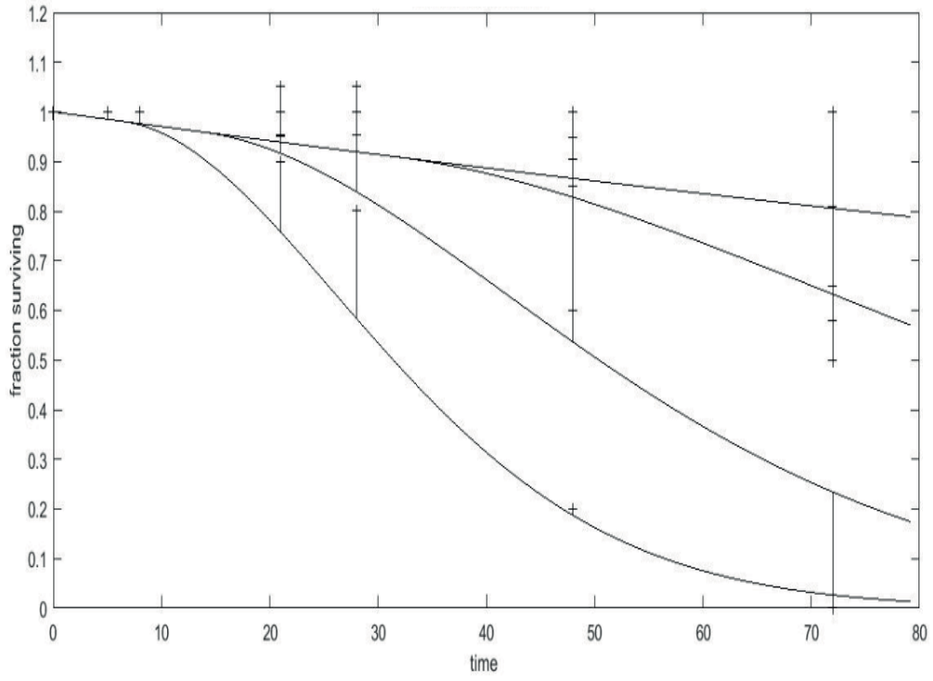


Figure S2. Model fit to dimethoate survival data (+ symbols). Each line represents a different concentration, although for visual clarity, some concentrations have been removed. Fig. S2a shows the model fit to the data without microplastics, fig. S2b shows the model fit to the data with microplastics.

The estimated parameter values are identical with and without microplastics (as could be expected as there are no differences in the survival matrices (see the results section of the main text). In addition, the value found for the No Effect Concentration in this research is in perfect agreement with an earlier estimate of 0.63 µM (Baas et al., 2016). LC_x values were calculated (Table S6) and compared to literature values (section S3.1.).

Table S3. Estimated parameter values for dimethoate with and without microplastics. Where present, numbers in brackets represent 95% confidence intervals.

Experiment	**BKR (hr^{-1})**	**NEC (mg l^{-1})**	**NEC (µM)**	**k_r (mg l^{-1} hr^{-1})**	**k_r (µM hr^{-1})**	**k_e (hr^{-1})**
Dimethoate without microplastics	1.7E-04	0.147 (0.101)	0.64 (0.44)	0.0053 (0.0039)	0.023 (0.017)	0.011 (0.009)
Dimethoate with microplastics	2.7E-03	0.105 (0.039)	0.46 (0.17)	0.023*	0.1*	0.004 (0.001)

* fixed in model

S3.1.2. Deltamethrin

As there was a large discrepancy between nominal and actual exposure concentrations for deltamethrin, the nominal chemical exposure concentrations cannot be used to inform the parameters of the model and obtain a reliable estimate of deltamethrin toxicity. We therefore needed to carry out reverse modelling based on known toxicity data, to allow us to estimate actual exposure concentrations and toxicity within our experiment. An independent estimate of the parameter values can be carried out if we have at least three LC_{50} values at different points in time that can be taken from the available literature. In the US-EPA ECOTOX database (US EPA, 2017) we can find 24, 48 and 96 hr LC_{50} values for *Daphnia magna* exposed to deltamethrin (most of the reported data contain only one point in time and are therefore of no use for a TK-TD approach). There is a significant range in the 48 hr LC_{50} values in different publications (Toumi et al., 2013; Xiu et al., 1989), but the numbers presented here (Table S4) are in line with the general picture that emerges from the database. With these values a NEC, killing rate and elimination rate could be derived (Table S5). From these parameters, a model was fit using survival over time (including 96 h, beyond the scope of the test) and thus

extrapolating to a realistic exposure concentration range (table 1). LC_x values were calculated (table S7) and compared to literature values as validation of the concentration measurements (section S3.2.).

Table S4. Toxicity data for daphnia exposed to deltamethrin over a 96 hour time period (Xiu et al., 1989)

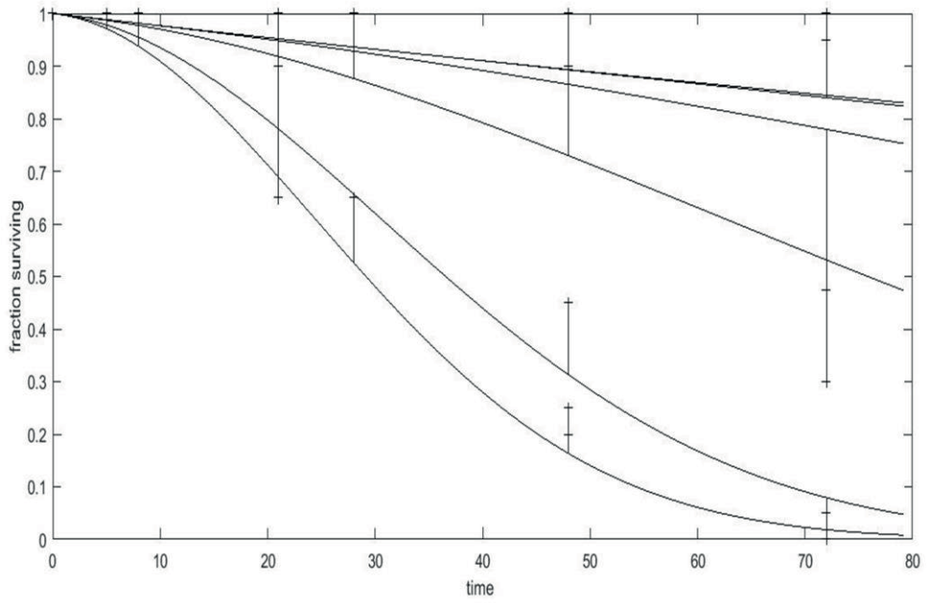
hr	**LC_{50} (ug l^{-1})**
24	0.13
48	0.038
96	0.01

Table S5. Estimated parameter values for deltamethrin.

Experiment	**BKR (hr^{-1})**	**NEC (ug l^{-1})**	**NEC (nM)**	**k_r (ug l^{-1} hr^{-1})**	**k_r (nM hr^{-1})**	**k_e (hr^{-1})**
Deltamethrin	1.7E-04	0.004	0.008	0.56	1.1	0.32

For the purposes of comparison to, and extrapolation from, other studies, for deltamethrin we can only focus on the data without microplastics. As the survival data shows no significant difference whether microplastics are present or not it is therefore reasonable to assume these are the same and therefore only one set of parameter values are presented (Table S5).

a



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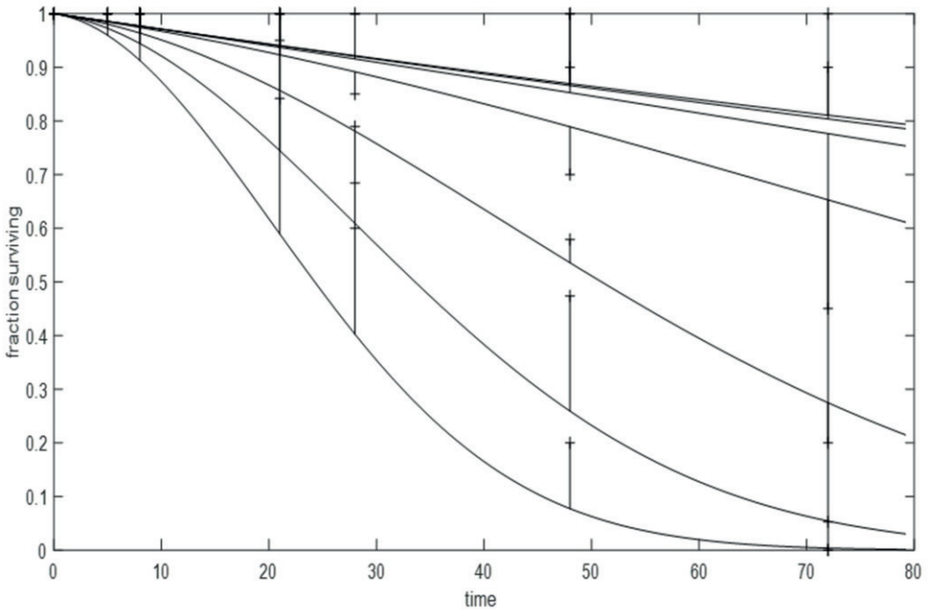

Fig. S3. Model fit to deltamethrin survival data (+ symbols). Each line represents a different concentration although for visual clarity, some concentrations have been removed. Fig. S3a shows the model fit to the data without microplastics, fig. S3b shows the model fit to the data with microplastics.

S4. Model-based LC_{50} values

S4.1. Dimethoate

The 48 h LC_{50} for dimethoate based on measured values was 1.22 mg l^{-1} which very closely resembles the 48 h LC_{50} value of 1.1 mg l^{-1} reported by Andersen et al. (2006). Beusen and Neven (1989) reported LC_{50} values of 1.7 and 2 mg l^{-1} for open and closed experimental systems respectively, values which are also very similar to our 48 h LC_{50}. Although all reported literature values are based on nominal concentrations, the limited difference between nominal and actual concentrations means these can be accurately compared.

Table S6. Modelled LC_x values for dimethoate at different time points based on calculated exposure concentrations.

LC_x (mg l^{-1})	Time (hr)			
	24	48	72	96
1	0.8	0.41	0.3	0.25
5	1.05	0.5	0.34	0.28
10	1.31	0.57	0.39	0.3
50	3.48	1.22	0.71	0.5
90	9.08	2.77	1.47	0.99

S4.2. Deltamethrin

The 48 h LC_{50} value of 0.046 µg l^{-1} as calculated by the model is comparable to the 48 h LC50 value of 0.12 µg l^{-1} reported on the deltamethrin safety data sheet (Sigma-Aldrich, 2017). The result is also within a similar range to that reported by Toumi et al. (2013) who calculated 48 h LC_{50} values of 0.32 µg l^{-1} and 0.63 µg l^{-1} based on measured concentrations, with variation dependent on the strain of *D. magna*. The modelled value for 96 h LC_{50} is 0.023 µg l^{-1}, which is in the same order of magnitude as the literature value of 0.01 µg l^{-1} calculated by Xiu et al. (1989). However these values should be treated with caution as these concentrations are approaching/exceeding the solubility limit of deltamethrin, and are often based on nominal concentrations.

Table S7. Modelled LC_x values for deltamethrin at different time points based on calculated exposure concentrations.

LC_x (µg l^{-1})	Time (hr)			
	24	48	72	96
1	0.024	0.015	0.012	0.011
5	0.032	0.018	0.014	0.012
10	0.040	0.021	0.016	0.013
50	0.118	0.046	0.029	0.023
90	0.321	0.109	0.064	0.046

Although 48 and 96 hour LC_{50}s for deltamethrin can be broadly compared to those of other studies, there is huge variability within the literature which suggests that determining LC_{50}s for deltamethrin is complicated, as solubility and LC_{50} can both be influenced by factors such as temperature, pH and vessel material.

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